compound eye of holometabolous insects like *Drosophila*, on the other hand, forms exclusively during the postembryogenesis, while the embryonic phase produces highly specialized larval eyes. We have begun to investigate the genetic basis underlying the evolutionary transformation of insect visual system development by studying eye development in grasshopper as model of primitive insect visual system development. Taking advantage of a recently developed strategy for systemic RNAi mediated gene knockdown in grasshopper nymphs, we imitated the temporal dynamics of *Drosophila* eye selector gene expression by transiently silencing the grasshopper orthologs of eyes absent (eya) and sine oculis (so) during nymphal development. Both regimens induced long-term but transient arrest of postembryonic eye development thereby mirroring the long intermission between the development of larval and adult eyes in holometabolous insects like *Drosophila*. These data unravel an inherent capacity of the eye gene network in primitive insects to partition visual system development into life cycle stage restricted phases. The results also highlight the lesser studied grasshopper as critical system to study ancestral mechanisms of stem cell facilitated postembryonic eye development in insects.

doi:10.1016/j.ydbio.2006.04.283

258
Genetic basis of pigmentation differences within and between *Drosophila* species

Patricia J. Wittkopp, Belinda K. Haerum, Emma Stewart, Alekhya Ratnala, Gizem Kalay, Gabriel Smith-Winberry
Univ. of Michigan, Ann Arbor, MI, USA

Drosophila pigmentation offers an excellent opportunity to investigate the developmental basis of evolutionary change. Pigmentation is one of the most frequently diverging traits in animals and its dispensability for viability makes it well suited to developmental analysis. In *Drosophila*, adult body color is composed of black, brown and yellow pigments. The location and abundance of each pigment are determined by expression of enzymes controlling biochemical reactions in the pigment synthesis pathway. Divergent *cis*-regulatory regions controlling expression of these enzymes contribute to phenotypic differences between species. To determine whether phenotypic changes within and between species are caused by similar developmental mechanisms, we study pigmentation differences between *D. novamexicana* and *D. americana* as well as variation within *D. americana*. *ebony* and tan encode enzymes that control opposite directions of a biochemical reaction in the pigment biosynthesis pathway and appear to contribute to both intra- and interspecific differences. Ebony expression has diverged between species, and a genetic association between the *ebony* gene and pigmentation suggests *cis*-regulatory changes are responsible for divergent expression. Expression and regulation of the *tan* gene are currently under investigation, although a genetic association has already been observed. Preliminary data indicate that the same genetic and developmental mechanisms generate pigmentation differences within and between species.

doi:10.1016/j.ydbio.2006.04.284

259
How to grow bigger legs: Insights from insects

Aleksandar Popadic, Najmus Mahfooz, Nataliya Turchyn
Wayne State University, Detroit, MI, USA

Differential enlargement of hind (T3) legs represents one of the hallmarks of insect evolution. Previously, we have observed that expression of the hox gene Ultrabithorax, Ubx, is localized only in T3 leg segments undergoing differential growth (compared to their foreleg counterparts). Here, we use RNA interference to elucidate further the molecular basis of hind leg divergence in two hemimetabolous insects, milkweed bug *Oncopeltus fasciatus* and the house cricket *Acheta domesticus*. Our results show that in both species, Ubx is regulating differential leg growth and determines which hind leg segments will be enlarged. We propose that diversification of T3 legs resulted from wide, species-specific regulatory changes in the spatial and temporal expression of Ubx. More specifically, the novel acquisition of Ubx expression in distinct hind leg segments provided positional information as to which regions of hind legs will be enlarged. At the same time, evolution of Ubx regulation also included changes in the timing of its expression during embryonic development. As a consequence, the degree of leg enlargement can be directly correlated to the duration of Ubx expression—the longer the expression, the bigger the affected segments. This study illustrates how evolution of even complex phenotypes, such as leg size, may be governed by selection in the regulation of a single selector gene.

doi:10.1016/j.ydbio.2006.04.285

260
The developmental and molecular basis of allometry in *Drosophila*

Alexander W. Shingleton [1], Jennifer A. Brisson [2], David L. Stern [2]
[1] *Michigan State University, East Lansing, MI, USA*
[2] *Princeton University, Princeton, NJ, USA*

Allometry is the scaling relationship between the size of an organism and the size of its constituent parts. Despite its obvious developmental and evolutionary importance, very little is known of the mechanisms that regulate allometries. Here, we look at one particular type of allometry—that created by rearing *Drosophila* under different nutritional conditions. *Drosophila* larvae that are fed increasingly suboptimal diets eclose into increasingly small adults with increasingly small body parts. Surprisingly, however, the male genitals remain approximately the same size under a range of nutritional conditions. The genitals therefore maintain a different allometric relationship with the body than other

structures. The insulin-signaling pathway is known to regulate growth with respect to nutrition, and suppression of the insulin receptor has less of an effect on the size of the genitals than it does on the wing, the maxillary palp, and the leg. Genetic and micro-array data suggest that the unusual allometric relationship between the genitals and the body is explained by differential expression of insulin-pathway genes in the genitals relative to other body parts. Organ-specific regulation of the insulin-signaling pathway may therefore be a general method by which animals maintain the size of key structures under variable nutritional conditions.

doi:10.1016/j.ydbio.2006.04.286

261
Cardiac arterial pole development is conserved in vertebrates

Margaret L. Kirby [1], Adrian Grimes [2]
[1] *Duke University, Durham, NC, USA*
[2] *Medical University of South Carolina, Charleston, SC, USA*

The arterial pole of the heart consists of the myocardium and smooth muscle that meet at the ventriculoarterial junction. Both cell types are generated by splanchnic mesoderm caudal to the outflow tract. The outflow tract moves caudally across this field as cells from the splanchnic mesoderm are added. Following the addition of myocardial cells to the prospective arterial pole, a second wave of smooth muscle cells is added. Even though the zebrafish has an undivided outflow tract, the arterial pole is formed at the ventriculoarterial junction where ventricular myocardium joins arterial smooth muscle at the base of a structure commonly called the bulbus arteriosus in teleosts. Our study was designed to determine whether development of the arterial pole of the zebrafish heart is conserved. Single cell zebrafish embryos were injected with caged rhodamine and cells were uncaged just prior to gastrulation. The embryos were allowed to develop to 72 hpf. The arterial pole was marked with DAF-2DA and tropomyosin to visualize the smooth muscle and myocardium. Both myocardial and smooth muscle cells in the arterial pole were found to arise from common progenitors located in the blastoderm in the vicinity of, but distinct from, ventricular progenitors. As in chick and mouse, normal arterial pole development is dependent on tbx1 (van gogh mutant), and FGF8 signaling (acerebellar mutant). These data provide the first evidence for evolutionarily conserved origin and development of cells that form the arterial pole of the vertebrate heart.

doi:10.1016/j.ydbio.2006.04.287

262
Hox genes and development of paired fins in teleost: An alternative view

Dae-gwon Ahn, Robert K. Ho
Univ. of Chicago, Chicago, IL, USA

Recent advances in molecular genetics have shown that pectoral and pelvic fins of teleost fishes are homologous structures to the fore- and hindlimbs of tetrapods, which utilize common sets of highly conserved developmental regulator genes for their growth and patterning. However, in contrast to the expression of many of the genes including Shh, Fgf8, Tbx4/5, which is mostly identical between limbs and fins, expression of Hox genes has been hypothesized to be quite different between fins and limbs. In particular, in contrast to the dynamic tri-phasic expression of Hox genes seen in tetrapod limbs, expression of hox genes in teleost fins has often been described as recapitulation of only the early parts of Hox expression within tetrapod limbs. Here, we show that a more detailed examination of the expression of posterior hox genes during pectoral fin development in zebrafish indicates the presence of at least three phases of hox gene expression which are similar but not completely identical to those seen in tetrapod Hox genes. Our results suggest that conservation of developmental mechanisms underlying formation and patterning of paired appendages between teleosts and tetrapods might be more extensive than previously thought.

doi:10.1016/j.ydbio.2006.04.288

263
Differential partitioning of paralog group 2 *Hox* gene expression within the *Osteichthyii*

Jean-Luc Scemama, Pierre Le Pabic, Edmund J. Stellwag
East Carolina University, Greenville, NC, USA

Hox cluster evolution, particularly those events related to an actinopterygian-specific genome duplication, have generated variation in the complement of *Hox* paralogous group 2 (PG2) genes within the *Osteichthyii*. Both the tetrapods and the ostariophysans, as represented by zebrafish, possess only two *Hox* PG2 genes, *hoxa2* and *b2* (tetrapods), and *hoxa2b* and *b2a* (zebrafish). In the acanthopterygians, however, all species characterized to date have three *Hox* PG2 genes (*hoxa2a*, *a2b*, and *b2a*). We have previously demonstrated that striped bass *hoxb2a* gene fails to be expressed in the pharyngeal arches, indicative of an evolutionary divergence between striped bass and other osteichthyians. Here, we report the expression analysis of striped bass *hoxa2a* and *hoxa2b* genes. We demonstrate that both genes are expressed in the developing hindbrain and the pharyngeal arches albeit with different spatio-temporal distributions relative to one another. We show that the striped bass *hoxa2a* gene expression pattern is similar to the overall expression pattern described for the *hoxa2* genes in the tetrapod lineage and for the *hoxa2b* gene from zebrafish. It is notable that the pharyngeal arch expression pattern of the striped bass *hoxa2a* gene is more divergent from its sister paralog, *hoxa2b* than from the zebrafish *hoxa2b* gene. These results associated with a comparative genomic analysis of the promoter region of these PG2 genes suggest that evolution-